What is a Hymenophore Subroutine in Fruiting Morphogenesis? - Evidence from a Hymenophore-less Mutant of *Coprinus cinereus*

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Introduction

A mushroom fruit body is a result of co-ordinate tissue development (Chiu & Moore, 1996; Moore, 1998). The spatial and temporal morphological pathway is a taxonomic characteristic of a species although developmental plasticity has been reported in many species too (Chiu & Moore, 1996; Moore, 1998). The convenience of using the haploid but dikaryon-phenocopy *AmBm* strains enable the recessive morphological mutants to reveal its controlled phenotype (Bourne *et al.*, 1996; Pukkila, 1996). Morphological mutants have been examined to dissect the morphogenetic pathways using model mushroom *Coprinus cinereus* (Takemaru & Kamada, 1971 & 1972; Kanda & Ishikawa, 1986; Chiu, 1988; Bourne *et al.*, 1996; Muraguchi & Kamada, 1998). A hymenophore-less mutant is the most interesting as hymenophore is for formation of sexual tissue and its maturation with the culminate of meiosis and sporulation, followed by spore discharge. Missing the differentiation of this tissues leads to the enormous waste of resources. Like other organisms, cell death may be a constructive use during development; Morphogenesis can require the removal of tissue as well as tissue growth and the cell death responsible for this must be controlled in time and position. This is programmed cell death. Thus, the examination of the hymenophore-less mutant enables to determine whether programmed cell death is involved and the definite pathway and architecture of a hymenophore.

Characterization of the Hymenophore-less Mutants

Several isolates were picked up after induced mutagenesis. They showed different colony morphology or fruit body morphology from that of the parent. Two hymenophore-less mutants were obtained and they were genetically different as verified by DNA fingerprints.

	Morphology		DNA Fingerprint using arbitrary or random primer				
	CM	MM	M13S	OPAA20	M13RS	EcoRI ext	Gal K54
AmBm	1	1	1	1	1	1	1
cw2	2	2	2	2	2	2	2
cw20	2	2	3	1	2	3	3
cw22	1	1	4	1	3	nd	nd
cw35	1	3	5	4	4	nd	nd
cw37	1	1	6	2	5	nd	nd
cw56	3	1	7	3	6	nd	nd

ILLUSTRATIONS

AmBm Parent Colony Morphology on MM DNA Fingerprints

Fruit body Morphology

Except its shortened stipe, the *AmBm* parent showed normal spore maturation, and the pileus expanded and shed off the black spores (Fig. 1). The primordia produced by the mutants were spherical, oval and sometimes with a grove appeared in the apical centre (Fig. 2). Both cw2 and cw20 mutants had a clear defined 'knob' indicating the presence of the pileus apex. In the larger fruits, veils cells were less obvious and were not deciduous as in normal parental fruits. Upper portion of presumably pileus region expanded and caused random cleavage but not splitting of the apical region, giving a 'crown-like' appearance. Lamellae were never found. Lower portion of presumably stipe was rather short, slender and sometimes curved, failed to fully erect the expanded pileus. In addition, no pileipellis was found, and veil cells were less developed (not swollen and elongated). Etiolated fruit bodies were observed in plate cultures by the parent and the mutants when incubated in darkness.

The Major Defect is the Failure to Form Hymenophore.

Implications of the Hymenophore-less Mutation:

1. Hymenophore Formation is Not Initiated by Cell Death

Mutant without lamellae and nor well-defined veil cells

Umar & Van Griensven (1997c) have suggested that there may be an involvement of cell death in the formation of the very first gill spaces in *Agaricus bisporus*. In the initials, hyphae extend in every direction so demarcated surfaces can only arise if pre-existing hyphae which cross the boundary before it is established can be severed.

Lu (1974, 1991) claimed that the gill cavities in *Coprinus* arise as a result of a cell disintegration process which he called a programmed cell death. What he observed are the multivesicular and membranous residual bodies in cells of the gill cavities on chemically fixed and processed TEM specimens. He proposed that cell disintegration to account for the deciduous veil and the separation of the stipe from the gills by a clear zone. Therefore, what he stated in the original article is on FREEing the gills from the stipe by cell disintegration and not exactly on the formation of gill cavities (the region between 2 neighbouring palisade layers of opposing gills). Further, many studies had dismissed the observation of cytoplasmic inclusions as fixation artefacts. Rosin & Moore (1985) could not find any sign of cell disintegration/cell death. Similarly fruit body development in *Coprinus* has also been examined in LM level by Reijnders (1963, 1979). In Rosin & Moore study, some cells are arranged and become the palisade layer when a gill cavity is finally formed. They named it the organizing centre for gill differentiation. When branches of determinate growth are formed opposing one another as a pair of palisade cell plates, they form an incipient fracture plane. This plane can be opened out into a cavity when the expansion of the underlying tissue puts tension across the 'fracture' and pulls the palisades apart (Moore, 1994).

In the hymenophore-less mutants, pileus expansion and mechanical stress to cause cell disintegration and death create space. However, no hymenophore is formed. Thus, this study would rather support the concept that a gill organiser must be present (Moore, 1994, 1998).

2. The Pleiotrophic Effect of the Mutation: Effect on Stipe Elongation

Hagimoto & Konishi (1959) and Gruen (1963) found an association pileus and the bends of the stipe in *Agaricus bisporus*. Experiments with *Coprinus* spp. (Borriss, 1934; Eilers, 1974; Gooday, 1974a; Cox & Niederpruem, 1975) suggest that the pileus is necessary only at the initial stages of fruit body development. After the stipe reaches 1/8 to 1/4 of its mature length, it can continue growth and show gravitropic responses without the pileus. At the later stage, Gooday (1974a) claimed that stipe elongation in *C. cinereus* did not require connection with the pileus suggesting that elongation is autonomous and endotrophic. However, Hammad *et al.* (1993a) demonstrated that intact fruit bodies elongated about 25% more than decapitated ones. Numerous studies seem to imply that extracts or diffusates of the pileus can stimulate growth of the stipe (Novak Frazer, 1996). As there is no hymenophore formation in the hymenophore-less mutants, growth factors/hormones were not produced to further stimulate stipe growth. Also, the pileus expansion created a stress. These fruits have shortened and slender stipe which might be curved.

3. The hymenophore subroutine

Well-defined pileipellis in AmBm parent (Left) but absent in mutant (Right). Veil cells are less differentiated in mutant.(top right).

Reijnders (1948, 1963, 1979) has stressed the contribution from the veil and pileipellis (the 'epidermis' of the pileus towards a mature fruit body form and shape. The most highly differentiated cells are found at the boundaries of tissue regions. In this case, this is the pileipellis. Clemencon (1997) reports various architectures of pileipelles. No differentiation of the pileipellis to well mark the outermost loose and deciduous swollen and enlarged veil cells from the inner closely packed pileus context, pileus trama and lamellae was revealed. The veil cells remained as loose thin cells in the mutant. Thus, veil cells and pileipellis are the secondary characteristic features of a hymenophore subroutine in addition to the appearance of lamellae.

Fruiting Development is a Co-ordination of Independent Subroutines.

Other Mutants Showed Similar Morphological Abnormality

The two hymenophore-less mutants were characterized by distinct DNA fingerprints and physiological properties. Recently a recessive allele-giving rise to similar phenotype was recovered from a field isolate in Japan (Muraguchi & Kamada, 1998). The defect in the Japanese isolate was owing to deletion of the promoter extending into the 5' region of a gene named *ich1* which encodes a novel protein containing nuclear targeting signals (Muraguchi & Kamada, 1998). In normal fruit body development, such transcript is specific for pileus. The abundance of the transcript decreased as basidiospores were produced (Muraguchi & Kamada, 1998). Further studies are needed to identify the genetic and molecular basis of the hymenophore-less mutants examined in this study.

Key References

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